

Detection of 2J5 in Formalin-Fixed, Paraffin-Embedded, Mouse Tissue

Reagents:

[1X Automation Buffer](#)
[3% Hydrogen Peroxide](#)
[Antibody Diluent](#)
[Citrate Buffer](#)
[DAB Chromagen](#)
[Hematoxylin](#)

Antibody Dilutions:

[Avidin Biotin Blocking Kit](#)
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog: #SP-2001

[Kit: Vector Elite Rabbit IgG ABC kit](#)
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # PK 6101.

*The Vector Rabbit Elite Kit contains solutions needed to make the secondary and label antibodies

[Primary Antibody Rabbit anti-2J5](#)
Antibody provided by: Dr. Darryl Zeldin, NIEHS

[Negative Control](#)
Pre-immune provided by: Dr. Darryl Zeldin, NIEHS

Staining Procedure

Positive Control Tissue: Heart, Pancreas ->Islets of Langerhans. Internal tissue positive control are autonomic ganglion.

Stain Localization: Cytoplasmic

Deparaffinize and hydrate slides through the following solutions.

Xylene	2 times	5 minutes
100% EtOH	2 times	3 minutes
95% EtOH	2 times	3 minutes
1X Automation Buffer	2 times	5 minutes

1. Quench endogenous peroxidase by placing slides in 3% hydrogen peroxide for 15 minutes.

2. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

3. Perform Heat Induced Epitope Retrieval using a microwave oven.

Unmasking Techniques

Place a full rack of slides in a container containing 250ml of 1X citrate buffer.

Microwave for 5 minutes at level 3.

Cool for 1 minute (Add 50 mls citrate buffer to the container).

Microwave again for 5 minutes at level 3. Temp. _____

Remove the slides from the microwave oven and allow to cool 10 minutes at room temperature.

Rinse slides in distilled water for 3 min.

4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

5. Apply block from Vector Elite kit for 20 min.

Exp Date _____ New Kit yes / no

DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

6. Apply Avidin/Biotin block

Lot# _____ Exp Date _____ New Kit yes / no

Apply avidin block - 15 min @ RT.

Quick rinse in 1X AB.

Apply biotin block - 15 min @ RT.

Wipe excess block.

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

7. Apply primary antibody 2J5 at a 1:2500 dilution and incubate for 1 hr.

Lot#_____ Aliquoted yes / no Date Aliquoted_____

For negative control slides, use pre-immune at a 1:2500 dilution for 1 hr.

Lot#_____ Exp Date_____

8. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

9. Apply secondary antibody from Vector Elite kit for 30 mins.

11. Apply Label antibody from Vector Elite Kit and incubate for 30 minutes.

Lot#_____ Exp Date_____ New Kit yes / no

12. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

13. Apply liquid Dako DAB Chromagen for 6 minutes in the dark.

(Add 1 drop of DAB per ml of substrate)

Lot#_____ Exp Date_____ New Kit yes / no

14. Rinse in tap water 3 minutes.

15. Counterstain with Modified Harris Hematoxylin for 30 seconds.

16. Rinse in tap water until water is clear.

17. Place slides in 1X Automation buffer for 1 minute with gentle agitation to blue slides.

18. Dehydrate through the following solutions.

95% alcohol	1 times	3 mins
100% alcohol	3 times	3 mins
Xylene	2 times	5 mins

19. Coverslip

Updated 10/28/03